SEQUENCE LISTING

<110> Miyawaki, Atsushi
      Sawano, Asako

<120> METHOD FOR MUTAGENESIS

<130> 11283-012001

<140> 09/920,922
<141> 2001-08-02

<150> JP 2000-237166
<151> 2000-08-04

<160> 9

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 720
<212> DNA
<213> Aequorea victoria

<220>
<221> CDS
<222> (1)...(717)

<400> 1
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atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg        48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1               5                  10                  15

gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc        96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc       144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                  40                  45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc       192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
    50                  55                  60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag       240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag       288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag       336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
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<pre>
              100   105   110

gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc   384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
         115   120   125

atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac   432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
     130   135   140

aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac   480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145   150   155   160

ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc   528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                 165   170   175

gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc   576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
             180   185   190

ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg   624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
         195   200   205

agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc   672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
     210   215   220

gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag   717
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225   230   235

taa   720

<210> 2
<211> 239
<212> PRT
<213> Aequorea victoria

<400> 2
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1   5   10   15
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
             20   25   30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
         35   ·   40   45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
     50   55   60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65   70   75   80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
             85   90   95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
         100   105   110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
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                 115                      120                      125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
         130                      135                      140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                      150                      155                      160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                 165                      170                      175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
             180                      185                      190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
         195                      200                      205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
         210                      215                      220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225                      230                      235
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<210> 3
<211> 717
<212> DNA
<213> Aequorea victoria

<220>
<221> CDS
<222> (1)...(714)

<400> 3
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atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca att ctt gtt     48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1               5                  10                  15

gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag     96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
             20                  25                  30

ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc    144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
         35                  40                  45

act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc    192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
     50                  55                  60

tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cag    240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65                  70                  75                  80

cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga    288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                 85                  90                  95

act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc    336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
             100                     105                     110

aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att    384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
         115                     120                     125
```

```
gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac       432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130             135             140

tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga       480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145             150             155             160

atc aaa gtt aac ttc aaa att aga cac aac att' gaa gat gga agc gtt      528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
            165             170             175

caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct       576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180             185             190

gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg      624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
            195             200             205

aaa gat ccc aac gaa aag aga gac cac atg gtc ctt ctt gag ttt gta      672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210             215             220

aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa             714
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225             230             235

taa                                                                   717
```

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<210> 4
<211> 238
<212> PRT
<213> Aequorea victoria

<400> 4
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1               5                   10                  15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
65                  70                  75                  80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
                100                 105                 110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160
```

```
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
                180                 185                 190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
                195                 200                 205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
                210                 215                 220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

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<210> 5
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetically generated primer

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gcactgcacg ccccaggtca gggtggt                                    27


<210> 6
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetically generated primer

<400> 6
gggcggactg gtagctcagg tagtgg                                     26


<210> 7
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetically generated primer

<221> misc_feature
<222> 9, 10, 11
<223> n = a, t, g, or c

<400> 7
gcggactgnn ngctcaggta g                                          21


<210> 8
<211> 239
<212> PRT
<213> Aequorea victoria

<400> 8
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Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
  1               5                  10                  15
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
```

```
            20                      25                      30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                      40                      45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
        50                      55                      60
Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                      70                      75                      80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                      90                      95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                     105                     110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
            115                     120                     125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                     135                     140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                     150                     155                     160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
            165                     170                     175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                     185                     190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
            195                     200                     205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                     215                     220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225                     230                     235
```

<210> 9
<211> 239
<212> PRT
<213> Aequorea victoria

<400> 9
```
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
1               5                       10                      15
Val Glu Leu Asp Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly
            20                      25                      30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
        35                      40                      45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
        50                      55                      60
Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                      70                      75                      80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                      90                      95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                     105                     110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
            115                     120                     125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                     135                     140
Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
145                     150                     155                     160
Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
            165                     170                     175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
```

```
              180                    185                    190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
         195                    200                    205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
         210                    215                    220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225                    230                    235
```